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An Exact Analysis of the Multistage Model Explaining Dose–Response Concavity

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The traditional multistage (MS) model of carcinogenesis implies several empirically testable properties for dose–response functions. These include convex (linear or upward-curving) cumulative hazards as a function of dose; symmetric effects on lifetime tumor probability of transition rates at different stages; cumulative hazard functions that increase without bound as stage-specific transition rates increase without bound; and identical tumor probabilities for individuals with identical parameters and exposures. However, for at least some chemicals, cumulative hazards are not convex functions of dose. This paper shows that none of these predicted properties is implied by the mechanistic assumptions of the MS model itself. Instead, they arise from the simplifying "rare-tumor" approximations made in the usual mathematical analysis of the model. An alternative exact probabilistic analysis of the MS model with only two stages is presented, both for the usual case where a carcinogen acts on both stages simultaneously, and also for idealized initiation-promotion experiments in which one stage at a time is affected. The exact two-stage model successfully fits bioassay data for chemicals (e.g., 1,3-butadiene) with concave cumulative hazard functions that are not well-described by the traditional MS model. Qualitative properties of the exact two-stage model are described and illustrated by least-squares fits to several real datasets. The major contribution is to show that properties of the traditional MS model family that appear to be inconsistent with empirical data for some chemicals can be explained easily if an exact, rather than an approximate model, is used. This suggests that it may be worth using the exact model in cases where tumor rates are not negligible (e.g., in which they exceed 10%). This includes the majority of bioassay experiments currently being performed.

KEY WORDS: Cancer dose–response modeling; MVK model; multistage model; two-stage model; hazard functions; carcinogenesis.

1. INTRODUCTION

Upper confidence limits on unit risk estimates produced by the linearized multistage model are not intended to reflect all, or even the most important, sources of uncertainty. For example, they do not address the following uncertainties, which may well dominate statistical sampling errors:

Model uncertainty (is the multistage model appropriate for a given dataset?)

Interindividual parameter variability, resulting from the fact that pharmacokinetic and other parameters may be different for different individuals.

Interindividual stochastic variability, arising from the fact that even identical individuals exposed to identical dose histories will develop randomly-sized initiated cell populations, and thus will have randomly different risks from carcinogens that act on initiated cells.

This paper presents a new analysis of the two-stage model that addresses aspects of model uncertainty and interindividual stochastic variability. A useful modern statistical treatment of interindividual parameter variability, with techniques for estimating both the population distri-

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bution of a parameter vector that affects individual risk and the conditional individual risk given the value of the parameter vector, is presented by Mentre and Mallet.[1]

Three specific problems that challenge the usefulness of the traditional multistage (MS) model are the following:

(i) It does not appear to provide a useful fit to some important cancer dose–response datasets. For example, it cannot explain or describe cumulative hazard functions that are concave functions of dose (see Fig. 1 and the discussion of it in Section 2).

(ii) It ignores variability in the population distribution of individual risks.

(iii) It makes qualitatively incorrect mechanistic predictions at high doses. For example, the MS model treats the transition rates (of cells from one stage to the next) for different stages symmetrically (see Appendix). If all stage-specific transition rates are positive, then the MS model allows the cumulative tumor hazard to become arbitrarily large as the transition rate for any stage becomes arbitrarily large. Thus, the predicted tumor probability approaches 1 as any transition rate approaches infinity. However, the physical basis of the MS model implies a different conclusion: if only the transition rates in late stages are large, then the smaller transition rates at the earlier stages become rate-limiting. The cumulative tumor hazard and lifetime tumor probability then become insensitive to the late-stage, large transition rates.

This paper shows that all of these objections to the traditional MS model are attributable not to the model itself, but to the simplifying approximations usually made in analyzing it. The model can be analyzed without approximations, leading to an exact analytic solution different from the usual (polynomial cumulative hazard) expression assumed in most multistage modeling software.[2] In a separate paper, we present a simple derivation and expression of the exact solution for any number of stages in vector-matrix notation. This paper derives and interprets the exact solution for the special case of two stages, which suffice for many dose–response datasets. The exact solution corrects each of the preceding three problems, showing that the physical assumptions of the MS model are compatible with a variety of datasets (e.g., those with concave cumulative hazard functions) even when the approximate mathematical model is not.

2. CONCAVE CUMULATIVE HAZARD FUNCTIONS

A testable prediction of the MS model is that the cumulative hazard function $H(x)$ should be a convex (upward curving or linear) function of the dose, x. Specifically, the traditional MS model has the form

$$P(x) = 1 - \exp[-(q_0 + q_1x + \ldots + q_nx^n)]$$

or, equivalently,

$$H(x) = -\ln[1 - P(x)] = q_0 + q_1x + \ldots + q_nx^n$$

where x = dose, $P(x)$ = lifetime probability of tumor at dose x, $H(x) = -\ln[1 - P(x)]$ = cumulative tumor hazard at dose x, and q_i = parameters of the dose–response relation (to be estimated from data). It is assumed that the parameters satisfy the constraints

$$q_i \geq 0$$

This implies that $H(x)$ must be a convex (upward-curving or linear) function of x, since each of the functions x^n is convex function of x.

A simple test for determining whether a given dataset is consistent with the multistage family of models is therefore as follows: *If the MS model holds, then, when $H(x)$ is plotted against x, the resulting curve should be convex.* Schell and Leysieffer (1989, p. 1120) provide a formal statistical test for the hypothesis of concavity of dose–response curves. A significantly concave $H(x)$ curve would show that the algebraic form of the multistage model is inappropriate (misspecified) for the dataset, making estimates of its parameter values irrelevant. Unless the concavity of $H(x)$ can be explained away by a concave relation between administered dose and biologically effective dose — a real possibility when metabolic saturation occurs, as demonstrated by Travis *et al.*[3] for tetrachloroethylene, but not plausible for nonsaturating dose levels — then a significantly concave $H(x)$ curve must be interpreted as evidence that the MS model does not adequately describe the data. Other models must then be sought to describe the dose–response relation. When a concave dose–response relation is observed, doubt that the MS model is applicable may undermine confidence in its predictions and conclusions—including its conclusions about upper confidence limits, which are predicated on the correctness of the model.

Enough data points are available for some chemicals at doses well below metabolic saturation levels to make a useful plot of x vs. $H(x)$. One such chemical is 1,3-butadiene. Melnick *et al.*[4] (Table 16.5) reported the data points shown in Table 1 for the dose–responses of male B6C3F1 mice exposed to butadiene for 2 years. The second (bottom) number in each pair gives the fraction of mice that developed tumors within 2 years. [Above 200 ppm, an ecotropic retrovirus or other mechanism is activated that dramatically increases the incidence of lymphoid leukemias, leading to $P = 0.86$ at x

Table I. Dose–Response Data for Male B6C3F1 Mice Exposed to Low and Moderate Concentrations of Butadiene for 2 Years[a]

x (ppm)	0	6.25	20	62.5	200
$P(x)$	0.33	0.55	0.62	0.68	0.73

[a] *Source:* Melnick *et al.*[4]

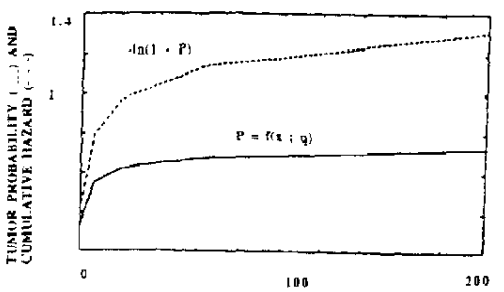

Fig. 1. A concave cumulative hazard dose–response curve for 1,3-butadiene.

= 625 (Melnick *et al.*).[4] Therefore, and also to avoid complications due to metabolic saturation, only the dose–response curve at concentrations at or below 200 ppm is considered here.] Figure 1 plots $H(x)$ against x for the data in Table I. The resulting curve is noticeably concave. The exposure concentrations are all well below the 1000-ppm level at which metabolic saturation is expected to become noticeable,[5] so a simple explanation of concavity by saturation of metabolism is not self-evident. Finding a "best-fitting" MS model for these data would be misguided, since the empirical dose–response curve appears to fall significantly outside the MS family.

If 1,3-butadiene were the only chemical exhibiting a significantly concave $H(x)$ function, it might be regarded as a curiosity, not worth modifying the MS model for. However, a recently completed study of isoprene (Battelle Columbus Labs, in press) shows a strongly concave $H(x)$ curve over a range of x values that includes 0, 10, 70, 140, 280, 700, and 2200 ppm (see Fig. 4). As shown in Fig. 4, some well-known chemicals are better described by concave rather than convex cumulative hazard functions when a family of models that includes both is fit to experimental data. As previously mentioned, Travis *et al.*,[5] as well as other authors, have found concave dose–response relations, although some of these may be explained by metabolic saturation (unlike the 1,3-butadiene and isoprene cases). Thus, empirical evidence suggests that realistic dose–response models must be flexible enough to allow for concave cumulative hazard functions. To this end, it is necessary to modify the traditional MS formula.

3. EXACT ANALYSIS OF A TWO-STAGE INITIATION-PROGRESSION MODEL

3.1. Qualitative Implications of the Multistage Model in an Initiation-Progression Setting

Before presenting an exact analysis of the two-stage MS model, it may be useful to illustrate and explain the main points in a simpler setting. This section considers an idealized initiation-progression experiment in which the roles and interactions of the initiation and progression stages can be studied separately. (The MS framework does not consider cell proliferation, so we discuss initiation-progression rather than initiation-promotion.) Animals are exposed to a pure initiator for L periods, followed by exposure to a pure progressor for T periods, after which terminal sacrifice occurs. As in the MS model, the initiator randomly mutates normal stem cells to create initiated cells. The progressor randomly transforms initiated cells into malignant ones.

If there are initially N normal stem cells and if the initiation rate is μ_1 mutations per normal cell per unit time during the first L periods and the progression rate is μ_2 mutations per initiated cell per unit time during the last T periods, then what is the probability that at least one malignant cell is formed prior to terminal sacrifice? The answer, according to the reasoning of the traditional MS model (reviewed in the Appendix) is as follows:

$$P = 1 - \exp(-\mu_1\mu_2 LTN)$$

(Armitage-Doll and MS formula)

This formula, well known as the Armitage-Doll formula for two stages, becomes the MS model when the linear μ_i are substituted into it. It implies the following conclusions:

(i) The cumulative tumor hazard, $H = \mu_1\mu_2 LTN$, is a convex (linear or upward-curving) function of the dose when the transformation rates are positive linear functions of dose, i.e., when

$$\mu_i = a_i + b_i x \quad \text{for } i = 1, 2, \text{ with } a_i > 0 \text{ and } b_i > 0$$

Figure 1 shows that this conclusion may be empirically false.

(ii) All individuals with identical parameters (i.e., with identical values for μ_1, μ_2, L, T, and N) have the same response probability, P.

(iii) μ_1 and μ_2 affect risk symmetrically.

(iv) If $\mu_1 LNT > 0$, then the P approaches 1 as μ_2 (and hence H) approach infinity.

A closer look at the model shows that *all four of these qualitative implications are false.* They result from simplifying approximations (the "rare-tumor" approximation discussed in the Appendix) made in the mathematical analysis of the model, rather than being logically implied by the model's assumptions.

A slight modification of the model makes it more realistic and helps to simplify its exact mathematical analysis. Henceforth, it will be assumed that *the number of normal stem cells remains approximately constant* throughout the experiment, e.g., due to homeostatic regulation of the normal stem cell compartment. (In the Armitage-Doll or MS model, by contrast, normal stem cells that become initiated are not replaced. Of course, if initiations are rare and N is large, the numerical difference in results made by assuming that N is depleted by initiation instead of assuming that N is conserved is negligible.) None of the conceptual points that follows depends on this modification (see the Appendix), but it makes the equations developed below more intuitive and easier to interpret.

3.2. Mathematical Analysis of the Model

The total number of initiated cells, denoted by I, formed by random mutations during the period of length L has a Poisson distribution with mean (and variance) given by

$$E(I) = \mathrm{Var}(I) = \mu_1 LN$$
$$= \text{expected number of initiated cells formed.}$$

The random number of malignant cells formed, denoted by M, is also a Poisson random variable, with mean and variance of

$$E(M) = \mathrm{Var}(M) = E(I)[1 - \exp(-\mu_2 T)]$$
$$= \mu_1 LN[1 - \exp(-\mu_2 T)]$$

(This follows from the fact that a binomially-sampled Poisson random variable is also Poisson with a mean equal to the mean of the original variable multiplied by the sampling probability; see e.g., Ross.[6]) Since each initiated cell becomes malignant with the same probability, namely, $1 - \exp(-\mu_2 T)$, binomial sampling applies.)

Now, consider the tumor probability P as $\mu_2 \to \infty$. The probability that at least one malignant cell is formed becomes the same as the probability that $I > 0$. The Poisson distribution of I implies that this probability is

$$\lim_{\mu_2 \to \infty} P = Pr(I > 0) = 1 - \exp[-E(I)] = 1 - \exp(-\mu_1 LN)$$

Thus, the limiting value of P may lie anywhere between 0 and 1, depending on the value of $\mu_1 LN$, even if $\mu_2 T > 0$, in contrast to the implications of the Armitage-Doll formula.

The exact probability that $M = 0$ also follows directly from its Poisson distribution:

$$Pr(M = 0) = \exp[-E(M)]$$
$$= \exp\{-(\mu_1 LN)[1 - \exp(-\mu_2 T)]\}$$

The total probability that at least one malignant cell is formed (interpreted as the "tumor probability") is therefore

$$E(P) = [1 - Pr(M = 0)]$$
$$= 1 - \exp\{-(\mu_1 LN)[1 - \exp(-\mu_2 T)]\}$$
(Exact formula for 2-stage model)

It is denoted by $E(P)$ to emphasize that it is the unconditional probability of tumor; it could also have been derived with more effort from the law of total probability, as

$$E(P) = E[E(P|I)] = \Sigma_{I \geq 0}[Pr(\text{at least 1 malignant cell}|I \text{ initiated cells}) * Pr(I \text{ initiated cells})]$$

Expressed in term of the cumulative hazard, the tumor probability is

$$E(P) = 1 - \exp(-H)$$

where H, the cumulative hazard, is given by

$$H = (\mu_1 LN)[1 - \exp(-\mu_2 T)] = E(I)[1 - \exp(-\mu_2 T)]$$

H has a direct physical interpretation as the expected number of malignant cells formed. This formula for H shows that it does not approach infinity as $\mu_2 T$ does, in contrast to the MS model. Instead, it approaches $E(I)$, explaining why the probability of tumor approaches a value less than 1. Also, as in the binomial case analyzed in the appendix, $H(x)$ need not be a convex function of x when

$$\mu_i = a_i + b_i x \quad \text{for } i = 1, 2, \text{ with } a_i \geq 0 \text{ and } b_i \geq 0$$

Figure 2 illustrates several possible shapes for $H(x)$.

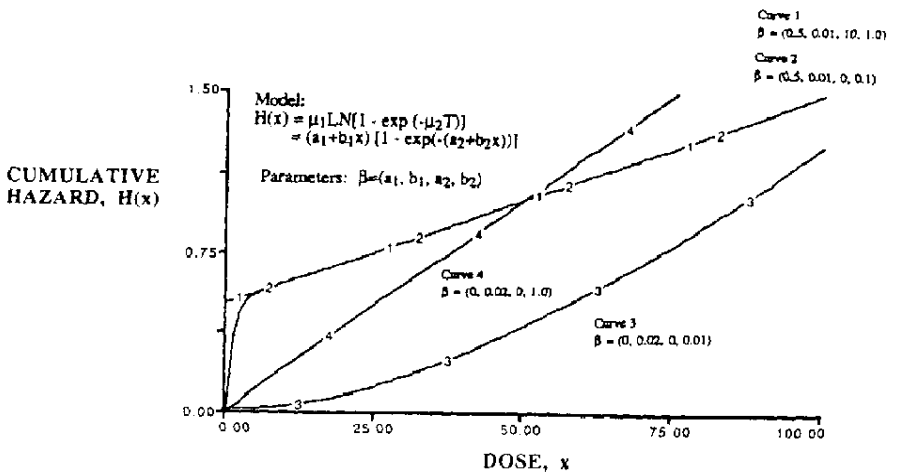

Fig. 2. Example dose–response curves for the exact two-stage initiation-progression model.

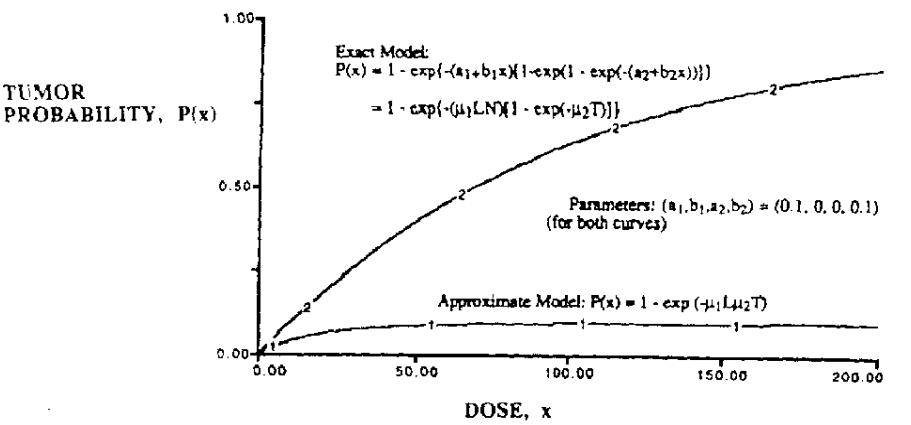

Fig. 3. The exact and approximate two-stage dose–response models may differ significantly at high doses.

From the preceding expression for $E(P)$, and recalling that $1 - \exp(-x)$ approaches x as x approaches 0, it is clear that the value of $E(P)$ approaches the MS model's value for P in the "rare-tumor limit" as $\mu_1 L$ and $\mu_2 T$ approach zero. However, this limit is inappropriate in many bioassay situations. Tumor incidence rates commonly exceed 20%, even in the absence of exposure (see Table I). Applying the *MS* model to analyze such data may yield misleading results. Figure 3 illustrates the potential discrepancy between exact and approximate model curves.

Finally, consider whether individuals with identical parameters and doses have identical risks. As just shown, the tumor probability P follows a nondegenerate frequency distribution in the population, i.e., it is *not* a constant [equal to $E(P)$] for all members of the population. Specifically, for an individual with I initiated cells, the (conditional) tumor probability is

$$P = 1 - \exp\{-I\,[1 - \exp(-\mu_2 T)]\}$$

For an individual with an unknown value of I, i.e., for whom I is a Poisson random variable with mean $E(I)$, the (unconditional) probability of tumor is just $E(P)$, since *individual risk is the mean of the population distribution of risks* when only the distribution, but not the actual value, of I is known. [This follows from the conditional expectation identity $E(P) = E(E(P \mid I)]$ (Ref. 6, p. 15, Ref. 7, p. 62). On the other hand, to a decision-maker concerned with the population distribution of risks, it may be important that some individuals (those

with high values of I) will be especially at risk of developing tumors if exposed to a carcinogen that increases μ_2. From this perspective, identically exposed individuals do not have identical (constant) risks, but rather *identically distributed* random risks. This distinction is also potentially useful in analyzing tumor count data from high-dose animal experiments, where there may be multiple tumors per animal. Among k identical exposed animals, the expected number of malignant cells created during an initiation-progression experiment, and also the variance of this number, are given by

$$kE(M) = k\mathrm{Var}(M) = k(\mu_1 LN)[1 - \exp(-\mu_2 T)]$$

To summarize this section, the Armitage-Doll formula for tumor probability (or the MS model, if the transition rates are specified to be linear functions of dose) is

$$P = 1 - \exp[-\mu_2 T^* E(I)] = 1 - \exp(-\mu_1\mu_2 LTN)$$
(Armitage-Doll/MS approximation)

The exact formula for the tumor probability is

$$P = 1 - \exp(-\mu_2 T^* I)$$
(Conditional tumor probability)

with expected value

$$E(P) = 1 - \exp\{-[\mu_1 LN][1 - \exp(-\mu_2 T)]\}$$
(Exact unconditional tumor probability)

For any specified values of $\mu_1 LN$ and $\mu_2 T$, the exact formula gives a smaller risk than the approximate one. The numerical difference between them approaches zero as $\mu_1 LN$ and $\mu_2 T$ both approach zero, but may be large for the range of $\mu_1 LN$ and $\mu_2 T$ values that are relevant to bioassay data analysis. For example, Fig. 3 shows the dose-response curves, i.e., the plots of $P(x)$ vs. x, from the exact and approximate models when

$$\mu_1 LN = 0.1 \text{ and } \mu_2 T = 0.1x$$

as x ranges from 0 to 200. Although the curves approach each other and essentially coincide for sufficiently small doses and tumor probabilities, they differ by more than a factor of 2 for tumor probabilities of 20% and more.

4. REGRESSION EQUATIONS FOR THE EXACT TWO-STAGE MODEL

The MS model is usually applied to situations in which a single chemical carcinogen is thought to act on different stages simultaneously, rather than having different chemicals act sequentially on different stages as in the idealized initiation-progression model. Instead of two distinct exposure periods there is only one, whose length will be denoted by T. This section derives the exact tumor probability (i.e., the probability that at least one malignant cell is created by time T) for such cases and shows that the main conclusions from the initiation-progression model extend to this setting.

The probability that at least one malignant cell is formed by time T may be derived as follows.

(i) The number of initiated cells created from time $t = 0$ to time $t = T$, denoted by I, is a Poisson random variable with mean $\mu_1 NT$.

(ii) Conditioned on the value of I, the arrival times of the initiated cells are independently and uniformly distributed over the interval from 0 to T (Ross, 1983, p. 37). The probability that an initiated cell created at time $t < T$ has not undergone a second (malignant) transition by time T is $\exp[-\mu_2(T - t)]$. Integrating over all times t in $(0, T)$ with respect to the uniform density $1/T$ shows that the total probability that an initiated cell created between 0 and T survives until time T without becoming malignant is given by the following quantity:

$$s = [1 - \exp(-\mu_2 T)]/\mu_2 T$$
$$= Pr\{\text{initiated cell does not become malignant}\}$$

(iii) The probability that none of the I initiated cells becomes malignant is s^I. Therefore, the conditional tumor probability (that at least one cell becomes malignant) is

$$P = 1 - s^I$$

(iv) The unconditional tumor probability (averaged over the conditional probabilities for all values of I weighted by their Poisson probabilities) is

$$E(P) = 1 - E(s^I) = 1 - \exp[-(1 - s)\mu_1 N]$$
(Exact two-stage model formula)

where the expectation follows directly from the moment generating function for a Poisson random variable. As in the initiation-progression model, this formula has a clear physical interpretation as

individual tumor probability = 1
$-$ exp[$-$(expected number of malignant cells formed)]

This completes the derivation of the tumor probability.

To apply these formulas to risk analysis, it is necessary to include dose explicitly and to develop methods for estimating the unknown quantities from data. Dose may be included as follows. Define the dose-dependent cumulative hazard at time T as

$$H(x) = (1 - s)\mu_1(x)NT$$

The formula for s and algebraic rearrangement yield

$$H(x) = [\mu_1(x)NT][\mu_2(x)T - 1 + \exp(-\mu_2(x)T)]/\mu_2(x)T$$

$$= N[\mu_1(x)/\mu_2(x)]\{\mu_2(x)T - 1 + \exp[-\mu_2(x)T]\}$$
$$= N[(a_1 + b_1x)/(a_2 + b_2x)]\{(a_2 + b_2x)T - 1 + \exp[-(a_2 + b_2x)T]\}$$

Now, recall that $H(x) = -\ln[1 - P(x)]$, where $P(x)$ denotes the tumor probability at dose x. Equating these two formulas for $H(x)$ gives the regression model

$$H(x) = N[(a_1 + b_1x)/(a_2 + b_2x)]\{(a_2 + b_2x)T - 1 + \exp[-(a_2 + b_2x)T]\}$$

As in the initiation-progression model, $H(x)$ may be either convex or concave, depending on the (nonnegative) values of its parameters. In this regression model, the quantities x and $P(x)$ (and hence $-\ln[1 - P(x)]$) are observed, although the observed value of $P(x)$, namely

$$P^*(x) = n(x)/N(x)$$

where $n(x)$ = number of responding animals exposed to x and $N(x)$ = total number of animals exposed to x, contains binomial sampling error. From the data points $P^*(x)$ for several dose groups, x, it is desired to estimate the parameters of the regression model.

It may appear that there are six unknown constants to be estimated, namely (N, a_1, b_1, a_2, b_2, T). However, N can be absorbed into the constants a_1 and b_1, since it multiplies them. Similarly, any fixed T can be absorbed into a_2 and b_2 (and into a_1 and b_1 in the first term, on multiplying by T/T). These simplifications reduce the quantal regression model to the following four-parameter family:

$$H(x) = [(a_1 + b_1x)/(a_2 + b_2x)]\{(a_2 + b_2x) - 1 + \exp[-(a_2 + b_2x)]\} \quad \text{(Regression model)}$$

The four parameters of this "reduced model" are scaled versions of the corresponding original parameters. They can be estimated by any of several nonlinear regression techniques,[8] e.g., using a maximum-likelihood or a least squares criterion.

A simple least-squares procedure is as follows. Given a data set of $[x, P^*(x)]$ pairs, transform the observed $P^*(x)$ values to obtain the corresponding values for

$$H^*(x) = -\ln[1 - P^*(x)]$$

Define the sum of squared errors for parameter vector $\beta = (a_1, b_1, a_2, b_2)$ and dataset D as

$$SS(\beta|D) = \Sigma_x[H^*(x) - H(x; \beta)]^2$$

where $H(x; \beta)$ represents the value for $H(x)$ predicted by the regression equation when parameter vector β is assumed. Use a nonlinear optimization software package (several are available in commercial statistical packages and in engineering software such as MATLAB) to find a value of β that minimizes $SS(\beta \mid D)$. The result is an ordinary least squares (OLS) estimate of β.

More sophisticated procedures may also be implemented quite easily using current commercial software. For example, a weighted least squares (WLS) algorithm would modify the $SS(\beta \mid D)$ criterion by weighting its individual terms, e.g., according to the reciprocals of their sample variances based on the numbers of animals in each group. Maximum likelihood estimation (MLE) would compute the likelihood of data set D for different values of the parameter vector β and choose a value of β to maximize it. Maximum *a posteriori* (MAP) Bayesian estimation would condition a prior probability density function for β on the observed data and select the value of β corresponding to the mode of the resulting *a posteriori* density function, assuming that it is uniquely identifiable from the data. In many applications, these different procedures lead to slightly different estimates of β but to very similar dose–response curves. (They also lead to different procedures for constructing confidence regions for β).[8] This paper will use the OLS procedure, as it is simple to understand and implement. A MAP approach to confidence region construction is developed in a separate paper.

Before turning to analyses of some example bioassay datasets, it is worth noting the following asymptotic properties of $H(x)$.

1. As $x \to 0$, $H(x) \to (a_1/a_2)[a_2 - 1 + \exp(-a_2)]$, assuming that $a_2 > 0$.

2. As $a_2 \to \infty$ with all other terms remaining bounded, $H(0) \to a_1$.

3. As $x \to \infty$, $H(x) \to b_1x$.

Thus, if only the no-dose ($x = 0$) and several high-dose groups are examined, it will be impossible to estimate b_2 [since it does not affect $H(0)$ or $H(\infty)$] or to uniquely estimate a_1 and a_2 [since several different combinations of their values give the same value of $H(0)$]. These difficulties are referred to as ill conditioning and nonidentifiability of parameters, respectively (Ref. 8, pp. 102–126). Even when intermediate values of $H(x)$ are available, the model may be modestly ill-conditioned, in that different choices of β approximately minimize $SS(\beta \mid D)$. However, to the extent that all β values that come close to minimizing $SS(\beta \mid D)$ give nearly identical $H(x; \beta)$ functions over the full range of x values, the lack of a uniquely identifiable "best" parameter estimate is irrelevant to risk assessment.

The example curves in Fig. 2 illustrate several of these points. (The same qualitative properties apply equally well to the solutions of the initiation-progression model and the two-stage model with simultaneous ef-

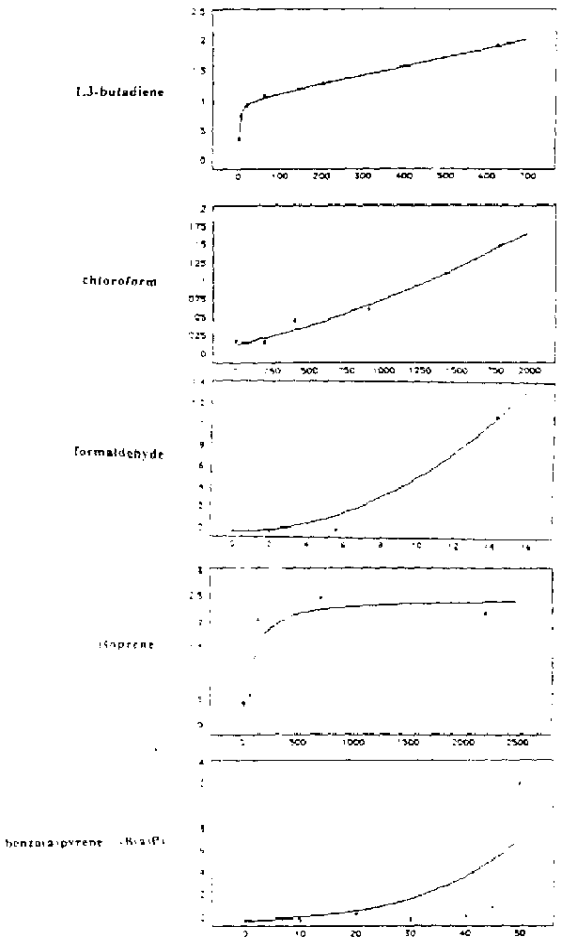

Fig. 4. Shapes of two-stage model dose–response curves for several example data sets.

fects on both transition rates; see Fig. 4.) Curve 1, generated by the parameter vector $\beta = (0.5, 0.01, 10, 1)$, is a nearly straight line with an intercept of approximately 0.5. From data points falling along this line, it would be impossible to determine the value of a_2, since changing its value from 10 to 30, for example, would produce no discernible difference in $H(x)$ or in the dose–response function (it is not rate-limiting). This is an example of ill-conditioning (many different parameter vectors produce almost the same $H(x)$ curve), but it is innocuous, since the risk at any given dose is insensitive to the value of a_2.

Curve 2, corresponding to the parameter vector $\beta = (0.5, 0.01, 0, 1)$, illustrates a greater difficulty. It essentially coincides with curve 1 at all doses above 10, but dives steeply toward zero at low doses. Although an observed data point at $x = 0$ (the control group) will discriminate between these two curves, manipulating the value of b_2 controls the location of the "knee" at which the high-dose slope (controlled by b_1) starts curving down toward the origin. A dose–response experiment that collects data only from exposure levels that bracket the "knee" (i.e., from the control group and from exposure groups with x values corresponding to the approximately linear segment) will make it impossible to estimate b_2, leaving the location of the "knee" and of virtually safe doses uncertain.

Curves 3 and 4, corresponding to $\beta = (0, 0.02, 0, 0.01)$ and $\beta = (0, 0.02, 0, 1)$, respectively, illustrate convex and nearly linear behaviors of the cumulative hazard function.

Table II. Fit of the Exact Two-Stage Model Regression Model to Bioassay Datasets for Five Chemical Carcinogens

Chemical	Variance explained by regression model (%)
1,3-Butadiene	99.9%
Chloroform	98.1%
Formaldehyde	97.3%
Isoprene	86.6%
$B(a)P$	52%

5. EXAMPLES ANALYSES FOR SEVERAL DATASETS

The exact two-stage model developed in the previous section has been fit to experimental bioassay data for several chemicals using the OLS technique. Figure 4 shows representative results for five chemicals: 1,3-butadiene, chloroform, formaldehyde, isoprene, and $B(a)P$. The formaldehyde, $B(a)P$, and chloroform datasets were taken from EPA's IRIS database, while the 1,3-butadiene and isoprene data were taken from the sources already cited. (Details of the datasets, including the dose metrics and the number of animals in each dose group, as well as the estimated parameter values, are available from the author or from these sources. Here, we only want to indicate the applicability of the exact two-stage model to some real datasets, rather than discussing these particular examples in depth.) Using the percentage of variance explained by the nonlinear regression model as a criterion, the fit of the model to the five datasets is summarized in Table II.

The two-stage model explains a substantial proportion of the variance in response frequencies in each case, as would be expected simply from the fact that dose is positively associated with response in both the model and the datasets. However, the quality of fit differs sharply among chemicals. It is worst for *B(a)P*. This might be expected *a priori* on biological grounds, since *B(a)P* is a potent promoter and the *MS* model (including the two-stage special case) ignores promotion based on cell proliferation. It is somewhat reassuring that the exact model is not flexible enough to provide a good fit to data that violate its mechanistic assumptions.

In summary, the exact two-stage model appears to provide useful fits to the bioassay data for the chemicals in Table II other than *B(a)P*. Since it is based on specific mechanistic hypotheses (and since it does not make simplifying mathematical approximations that would allow it to fit datasets that are inconsistent with its assumptions), it is to be expected that the model will be inappropriate for some chemical carcinogens—especially, for those that act solely or primarily by expanding the net growth rate of the initiated population. For such carcinogens, the modeling approach illustrated in this paper must be extended to obtain exact formulas for situations where dose-dependent birth–death parameters are important.

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APPENDIX: DERIVATIONS OF EQUATIONS

The Traditional Multistage Model with Two Stages

If there are initially N normal stem cells and if the initiation rate is μ_1 mutations per normal cell per unit time during the first L periods and the progression rate is μ_2 mutations per initiated cell per unit time during the last T periods, then the probability that a normal stem cell survives until terminal sacrifice without becoming malignant is

$$Pr(\text{cell is never initiated}) + Pr(\text{cell is initiated but not transformed})$$
$$= \exp(-\mu_1 L) + [1 - \exp(-\mu_1 L)][\exp(-\mu_2 T)]$$

The probability that a normal stem cell does become malignant is therefore

$$p = Pr(\text{a specific cell becomes malignant}) = 1 - \exp(-\mu_1 L) - [1 - \exp(-\mu_1 L)][\exp(-\mu_2 T)] = [1 - \exp(-\mu_1 L)][1 - \exp(-\mu_2 T)]$$

If $\mu_1 L$ and $\mu_2 T$ are sufficiently small (*the rare-tumor approximation*), then this expression is well approximated numerically as

$$p = \mu_1 L \mu_2 T = \mu_1 \mu_2 L T$$

The probability that at least one of the N cells becomes malignant, which is given exactly by the binomial probability

$$P = 1 - (1 - p)^N$$

is numerically well approximated, for sufficiently small p (and therefore for sufficiently small $\mu_1 L$ and $\mu_2 T$, by the formula

$$P = 1 - \exp(-Np) = 1 - \exp(-\mu_1 \mu_2 L T N)$$

If it is assumed that

$$\mu_i = a_i + b_i x$$

where x is the administered dose, then

$$P = 1 - \exp[-(a_1 + b_1 x)(a_2 + b_2 x) L T N] = 1 - \exp[-(q_0 + q_1 x + q_2 x^2)] \quad \text{(MS Model)}$$

where $q_0 = a_1 a_2 LTN$, $q_1 = (a_1 b_2 + a_2 b_1) LTN$, and $q_2 = b_1 b_2 LTN$. Since the a_i and b_i are nonnegative, the q_i will be nonnegative, too.

Exact Analysis of the Two-Stage Initiation-Promotion Model

The random number of initiated cells formed during the initiation period, denoted by I, has a binomial distribution with parameters N and $1 - \exp(-\mu_1 L)$. The conditional probability that none of them becomes malignant is

$Pr(\text{no malignant cells formed} \mid I \text{ initiated cells}) =$

$$[\exp(-\mu_2 T)]^I = [\exp(-\mu_2 TI)]$$

which has the unconditional expected value

$$\begin{aligned} &Pr(\text{no malignant cells formed}) \\ &= E[\exp(-\mu_2 TI)] \\ &= \{[1 - \exp(-\mu_1 L)]\exp(-\mu_2 T) + \exp(-\mu_1 L)\}^N \\ &= [\exp(-\mu_2 T) - \exp(-\mu_1 L)\exp(-\mu_2 T) + \exp(-\mu_1 L)]^N \end{aligned}$$

(This may be confirmed either by noting that the expected value term is essentially the moment generating function of the binomial distribution of I, or else by recognizing that the expression may be interpreted as the probability that, for each of the N cells, either no initiation occurs or initiation occurs but progression does not.) The probability that at least one malignant cell is formed is therefore

$$P = 1 - [\exp(-\mu_2 T) - \exp(-\mu_1 L)\exp(-\mu_2 T) + \exp(-\mu_1 L)]^N$$

The corresponding cumulative tumor hazard is

$$\begin{aligned} H &= -\ln(1-P) = \\ &-N^* \ln[\exp(-\mu_2 T) - \exp(-\mu_1 L)\exp(-\mu_2 T) \\ &+ \exp(-\mu_1 L)] \end{aligned}$$

When expressed as a function of dose, x, assuming that

$$\mu_i = a_i - b_i x \quad \text{for } i = 1, 2, \text{ with } a_i \geq 0 \text{ and } b_i \geq 0$$

this function may be concave (e.g., choose $\mu_1 L = 0.01$ and $\mu_2 T = 0.01 + 0.1x$) or convex (choose $\mu_1 L = \mu_2 T = 0.01x$). Thus, solving the model exactly instead of approximately (via the rare-tumor approximation) shows that the dose–response relation for cumulative tumor hazard vs. dose may be concave, contrary to the predictions of the approximate model.

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